IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

SCHEHLMANN et al — Atty. Ref.: 4804-6; Confirmation No. 3196

Appl. No. 10/593,487 — TC/A.U. 1614

Filed: October 31, 2006 — Examiner: STONE, Christopher R.

For: COMPOSITION COMPRISING AN HDAC INHIBITOR IN COMBINATION WITH A RETINOID

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 CFR §1.132

I, **Jochen Klock** hereby declare as follows:

1. That I am an inventor of the subject application, a citizen of Germany and that my address is as stated in my Declaration under Rule 63 of record in the subject application.

2. That I am employed by DSM Nutritional Products, B.V. (previously Roche Vitamins Ltd. and F. Hoffmann-La Roche Ltd.), Basel, Switzerland and have assigned my rights in the subject application to DSM IP Assets B.V.

3. Attached hereto is my Curriculum Vitae listing my publications in the field.

4. I have read and am familiar with the Official Action dated May 12, 2009 as well as the published international patent applications of Gudas et al WO 02/060430 A1 and Collier et al WO 01/10427 A2.

5. That the following experiments were conducted by me or under my direction and control:

An *in vitro* cell culture of human primary keratinocytes has been chosen to show the synergistic mode of action of Retinol and 4-phenyl butyric acid, respectively retinal and 2-phenyl acetic acid. This model is able to simulate the *in vivo* situation in human skin by studying differentiation processes (Poumay et al., 1999). To monitor such differentiation processes transglutaminase 1 (TG1) is a well known marker molecule (Polakowska et al., 1999).

Culture of epidermal keratinocytes

Epidermal keratinocytes were isolated from human foreskin biopsies and cultured in keratinocyte serum free medium (KSFM made by GIBCO) in a growth chamber with 37°C and 5 % CO_2. At the second passage, cells were transferred to 6 well plates and allowed to reach approximately 50% surface confluence.

Expression of transglutaminase 1 (TG1) by differentiating keratinocytes

Retinol was solubilized in ethanol/tetrahydrofuran and 4-phenyl butyric acid and phenylacetic acid were independently solubilized in DMSO. Retinol solutions were handled under yellow light conditions only. When keratinocyte cultures had reached the appropriate confluence, the KSFM medium was supplemented with 1.3 mM calcium, in order to induce keratinocyte differentiation and thus induce TG1 expression and the treatment was started with either retinol in concentrations of 1×10^{-9} M, 4-phenyl butyric acid 50.0 µM or phenyl acetic acid 0.5 µM alone or a combination of retinol plus one of the two substances. For every sample, medium and treatment substances were changed twice daily.

Seventy-two hours after the beginning of the treatment, cells were harvested and the RNA extracted. RNA was reverse transcribed into cDNA. Relative quantification of TG1 mRNA transcript levels in control versus treatment cultures were determined using multiplexed real time PCR analysis. Repression level is given as a normalized multiplicator relative to the non-treated test cell. The results are shown in table 1. Retinol, 4-phenyl butyric acid or phenylacetic acid alone showed only weak modulation of the terminal differentiation process in epidermal keratinocytes. Surprisingly in combination synergism emerges. The differentiation marker TG1 in human epidermal keratinocytes was more than 1.4 fold or 2.3 fold downregulated for the combination of retinol plus either 4-phenyl butyric acid or phenylacetic acid, respectively.

The enzyme transglutaminase 1 is a well-known and accepted differentiation marker in human epidermal cells. Cultures with keratinocytes expressing this marker are a model to simulate the differentiation process of the skin. Slowing this process down results in an improved epidermal thickness. Based upon my experience these studies show that when applied together, 4-phenyl butyric acid, or phenylacetic acid in combination with a retinoid slowed down the process.

Substance	*Concentration*	*Fold change in TG-1 expression*	*Synergistic change (total observed/ additive)*
Retinol	1×10^{-9} M	-1.47	
4-Phenyl butyric acid	50.0 µM	-0.09	
Phenylacetic acid	0.5 µM	+0.35	
Retinol + 4-phenyl butyric acid	1×10^{-9} M + 50.0 µM	-2.20	1.4x
Retinol ± phenylacetic acid	1×10^{-9} M + 0.5 µM	-2.57	2.3x

5. That the above information to the best of my knowledge is correct and accurate.

6. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 29.07.2009

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Curriculum Vitae

Dr. Jochen Klock

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I studies biology and received my Ph.D. in genetics and molecular biology at the University of Cologne in 2000.

My scientific career I started at the Max-Planck-Institute in Cologne and Freiburg with a special emphasis on genetics, cellular signaling and skin biology.

In 2001 I joined Roche Vitamins Ltd meanwhile known as DSM Nutritional Products and was heading the skin biology and skin penetration unit for Personal Care.

Since 2005 I am responsible for Portfolio Management in Personal Care covering the R&D segments for skin, sun and hair care.

In January 2008 I joined the New Business Development department and was appointed as Global Marketing Manager for Hair Care.

Publications

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Date: 29.07.2009 Signature: